

Adeno-associated virus (AAV) production

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 An abbreviated version of this protocol was published in eLIFE in Jul 2020

An adipokine feedback regulating diurnal food intake rhythms in mice

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Detailed protocol

All steps were essentially followed as outlined in the manufacturer's protocol (AAV Purification Standard Kit; Cell Biolabs Inc.;

<https://www.cellbiolabs.com/sites/default/files/VPK-140-aav-purification-kit.pdf>).

The following procedure was used for one 15 cm dish:

Production:

1. HEK 293T cells were passaged 2-3 times prior to transfection and then cultured until 70-80% confluency.
2. Co-transfect cells with the pAAV-DJ, pAAV-helper and the expression construct (Ca phosphate precipitation method).
3. After 60 hrs, replace medium with 2.5 mL TBS/1% Triton (TBS-T) and incubate for 3 min at room temperature. Gently shake the culture plate several times and harvest all media, including cells, in a sterile tube.
4. Centrifuge for 5 min at 3,000 rpm to pellet the transfected cells. Resuspend the cell pellet in 2.5 mL TBS-T.
5. Subject the cell suspension to three rounds of freeze/thaw cycles by alternating the tubes between a dry ice-ethanol bath and a 37°C water bath. Resuspend in 5 mL culture medium.
6. Collect the AAV supernatant by centrifugation at 10,000 x g for 10 minutes. Discard the pellet.
7. The viral supernatant was immediately purified.

Purification:

1. Add 1,000 U of benzonuclease (Millipore E1014) and incubate for 1 hr at 37°C.
2. Filtrate mixture through a 0.2-mm low-protein binding filter (Millipore Milliguard).
3. Load filtrate to AVB HiTrap sepharose columns (GE Healthcare) with a perfusion pump at a flow rate of 0.75 ml/min.
4. Elute AAV particles with a low-pH elution buffer (0.2 M glycine-HCl, pH 2.7; 5 mL)
5. Immediately neutralize with neutralization buffer (1 M Tris-HCl, pH 8.0; 5 mL).
6. Buffer-exchange to PBS and concentrate with Amicon Ultra centrifugal filter units (molecular weight cut-off of 10 kDa; Millipore - retain 100 µL).
7. Resuspend retained solution in 400 µL PBS
8. Reconcentrate if necessary (step 6)
9. Filter-sterilize by passing through a 0.2-mm low-protein binding filter.
10. Viral titers were determined with a commercially available qPCR-based titration kit (e.g., from Takara Cat. 6233).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Oster, H. (2023). Adeno-associated virus (AAV) production. Bio-protocol Preprint. bio-protocol.org/prep2121.
2. Tsang, A. H., Koch, C. E., Kiehn, J., Schmidt, C. X. and Oster, H. (2020). An adipokine feedback regulating diurnal food intake rhythms in mice. eLIFE. DOI: [10.7554/eLife.55388](https://doi.org/10.7554/eLife.55388)

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